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Hyperhomocysteinemia: a risk factor for placental abruption or infarction

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Abstract

Objective: To establish the prevalence of hyperhomocysteinemia in women with placental abruption or infarction. *Design:* Forty-six women with normal pregnancy outcome (controls) and 84 women with placental abruption or infarction (study group) were selected, and studied in the non-pregnant state. Homocysteine metabolism was investigated by a standardized oral methionine loading test. Hyperhomocysteinemia was defined as a concentration of fasting and/or postmethionine plasma homocysteine exceeding the estimated 97.5 percentile level of the controls. In the fasting state, the vitamin status was investigated by the measurement of serum and red cell folate, serum vitamin B12, and whole blood pyridoxal-5′-phosphate (PLP, an active form of vitamin B6). *Results:* Hyperhomocysteinemia was diagnosed in four controls (9%) and 26 women of the study group (31%, $P < 0.05$). The median concentrations of the vitamins studied were significantly lower in women of the study group as compared to the controls, except for red cell folate, where the median concentration was comparable in both groups. The median concentration of fasting plasma homocysteine, unlike post-methionine plasma homocysteine, was significantly higher in women who experienced placental abruption or infarction in their first pregnancy than in women who had the same event after one or more uncomplicated pregnancies. *Conclusion:* Hyperhomocysteinemia is associated with placental abruption or infarction.

Keywords: Homocysteine; Placental abruption/infarction; Folate; Vitamin B12

1. Introduction

Homocysteine is the demethylated derivative of the essential amino acid methionine (Fig. 1). Homocysteine is either transsulfurated via cystathionine into cysteine or it is remethylated to methionine [1]. The conversion of homocysteine to cystathionine is catalyzed by the enzyme cystathionine β-synthase (CBS), requiring pyridoxal-5′-phosphate (PLP), an active form of vitamin B6, as a cofactor. In humans, at least two pathways exist for the remethylation of homocysteine into methionine [1]. One of these reactions is dependent on folate and vitamin B12 (Fig. 1).

Defects in either the transsulfuration or remethylation pathway lead to accumulation of homocysteine resulting in hyperhomocysteinemia [1–3]. The most frequent cause of severe hyperhomocysteinemia is CBS deficiency, an autosomal recessive inherited disorder. Premature arteriosclerosis and thrombosis are the most life-threatening complications in these patients [4].

Heterozygosity for CBS deficiency and thermolabile methylenetetrahydrofolate reductase (MTHFR, see Fig. 1) cause moderately elevated levels of blood homocysteine [5–8]. Mild hyperhomocysteinemia is a well-known risk factor for premature vascular disease [9–11].

In a preliminary study, hyperhomocysteinemia was reported as a possible risk factor in women with recur-

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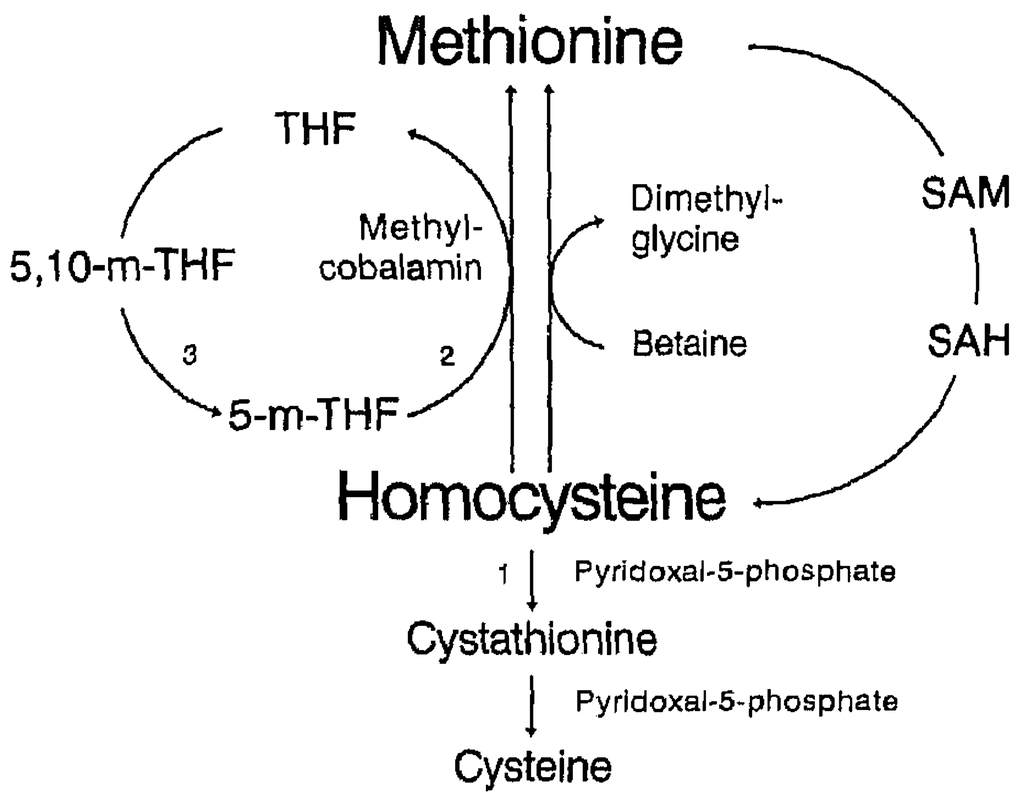

Fig. 1. Simplified scheme of methionine-homocysteine metabolism and the most important enzymes and related vitamins. (1) Cystathionine β-synthase; (2) 5-methyltetrahydrofolate homocysteine methyltransferase; (3) 5,10-methylenetetrahydrofolate reductase. SAM, *S*-adenosylmethionine; SAH, *S*-adenosylhomocysteine; THF, Tetrahydrofolate; 5-m-THF, 5-methyltetrahydrofolate; 5,10-m-THF, 5,10-methylenetetrahydrofolate.

rent spontaneous abortion or placental abruption [12]. Recently, the results of an extended study of hyperhomocysteinemia in women with unexplained recurrent early pregnancy loss were presented [13]. In the present study, we report the results of an extended investigation in 84 women with placental abruption or infarction.

2. Subjects and methods

2.1. Subjects

Placental abruption and placental infarction were defined by clinical, laboratory and histologic standards. The diagnosis of placental abruption was based on either the combined presence of a tender, hypertonic uterus and disseminated intravascular coagulation, and/or the histologic observation of a retroplacental hematoma with or without signs of infarction. Placental infarction was diagnosed if the placenta was characterized by circumscribed areas of villous necrosis combined with a stillborn fetus or a severe growth-retarded child, i.e. having a birth weight below the 10th percentile for gestational age. Data on clinical and laboratory features were collected by interview and hospital records. Histologic data were drawn from various pathologists' reports. Eighty-four women who were referred to the hospital because they had a history of placental abruption or infarction fulfilled the clinical, laboratory and histologic standards (study group). Forty-four women (52%) had experienced abruption of the placenta, whereas the remaining 40 women (48%) had suffered from placental infarction (as a first event). As controls, 46 women (aged 27–44 years) having at least one live-born child (range 1–4), and without a history of neural tube defect, (recurrent) spontaneous abortion, fetal death, fetal growth retardation or placental abruption, were recruited by public advertisement. All participants (n = 130) were generally healthy and had no evidence of diabetes mellitus, renal or liver dysfunction. The study was approved by the Ethical Committee of the University Hospital Nijmegen St. Radboud, Nijmegen, The Netherlands. Before participation informed consent was obtained from all subjects.

2.2. Investigation procedure

Homocysteine metabolism was investigated by a standardized oral methionine loading test. After an overnight fast, venous blood samples were collected to measure the concentrations of plasma homocysteine and blood vitamins (folate, vitamin B12, and PLP). Thereafter, L-methionine, 0.1 g (0.7 mmol) per kg body weight, was administered orally in 200 ml orange juice. All women used a standardised methionine-restricted breakfast and luncheon. No drinks, except for coffee and tea without milk were allowed during the test procedure. After 6 h, a venous blood sample was drawn to assay the postmethionine plasma homocysteine concentration. To minimize possible hormonal influences on methionine-homocysteine metabolism, the loading tests were performed about 1 week before the expected first day of the next menstrual period. Women were instructed not to become pregnant until completing the investigation procedure. They were not allowed to take oral contraceptives, hormonal and/or vitamin supplements, or other medication which could possibly interfere with methionine-homocysteine metabolism, for at least 3 months prior to the oral methionine loading test [14]. Women were tested at least 2 months after completing their last pregnancy (median time interval of the study and control group, 6 and 49 months, respectively)

Hyperhomocysteinemia was defined as a fasting and/or postmethionine plasma homocysteine concentration exceeding the estimated 97.5 percentile level of the controls.

2.3. Sample preparation and analysis

Blood samples for measurements of total homocysteine concentrations in plasma were drawn in ethylenediamine tetraacetate (EDTA) vacutainer tubes of 4 ml and centrifuged within 30 min at 3000 × g for 10 min. The plasma was separated and stored at −20°C. Total homocysteine concentrations were measured by high-performance liquid chromatography (HPLC) technique and fluorometric detection (detection limit 0.5 μmol/l; intra- and inter-assay coefficients of variation,

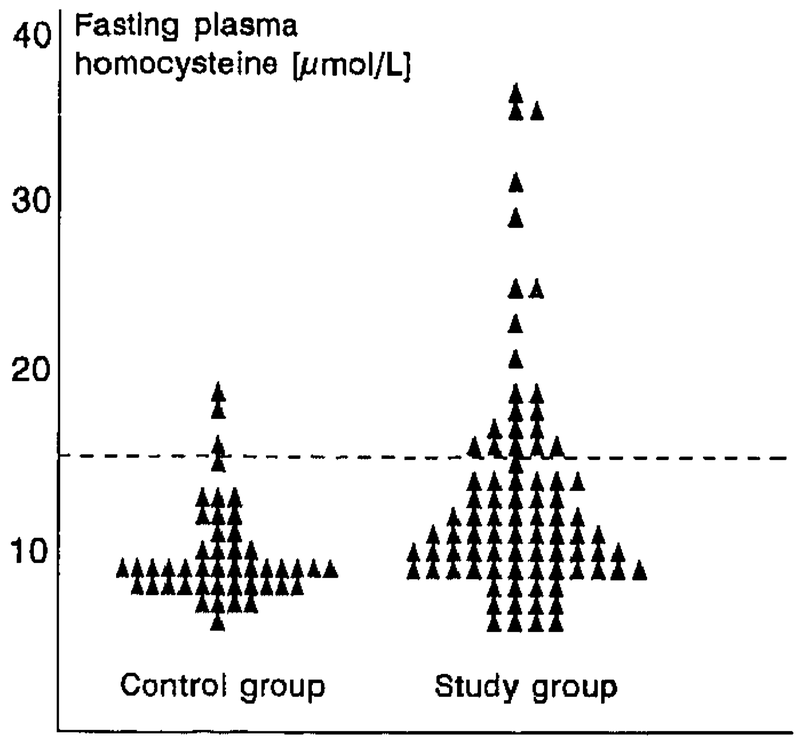

Fig. 2. Individual concentrations of fasting plasma homocysteine in women of the control ($n = 46$) and study group ($n = 84$).

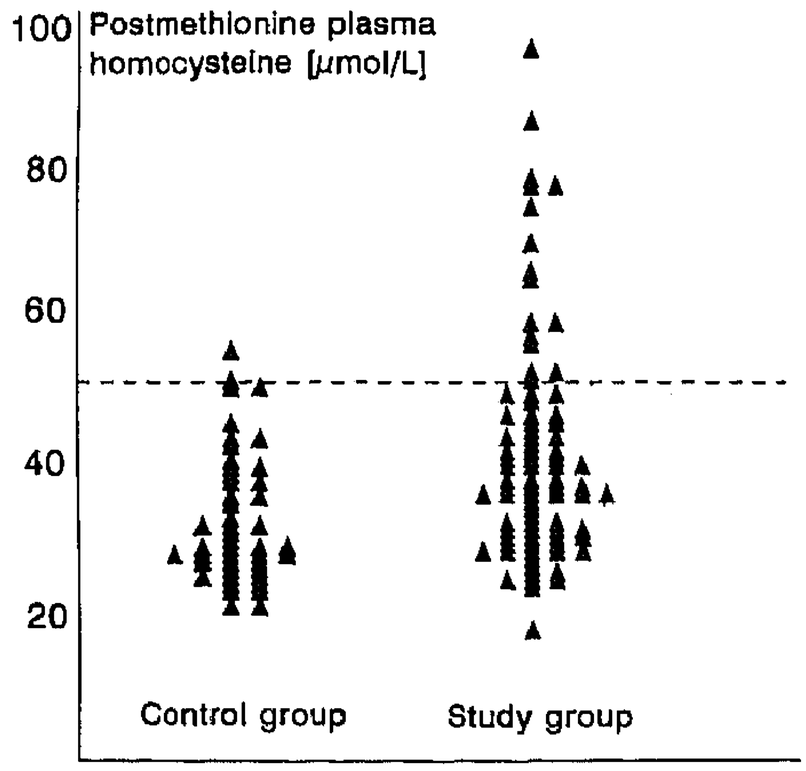

Fig. 3. Individual concentrations of postmethionine plasma homocysteine in women of the control ($n = 46$) and study group ($n = 84$).

both <5%) [15,16]. Dry and heparinized vacutainer tubes of 10 ml were used for collecting venous blood samples to assay the concentrations of folate (serum and red cells), vitamin B12 (serum), and PLP (whole blood). Folate and vitamin B12 concentrations were measured simultaneously with Dualcount SPB (solid phase boil) Radioassay (Diagnostic Products Corporation, Los Angeles, CA), as described previously [17]. Determination of PLP was performed by HPLC technique [18].

2.4. Data analysis

In controls, the 2.5 and 97.5 percentile levels of plasma homocysteine and blood vitamins were calculated as means ± 1.96 standard deviations (S.D.) after log transformation. In women of the study group, true 2.5 and 97.5 percentile values were established. Wilcoxon rank sum test was used to analyze the quantitative differences, and uncorrected chi-square test to analyze the proportional differences between the two groups studied. Spearman's rank correlation was used to measure the associations between variables. *P*-values were two-tailed, and $P < 0.05$ was considered statistically significant.

3. Results

Figs. 2, 3 depict the individual concentrations of fasting and postmethionine plasma homocysteine in the control and study group, respectively. In the control group, fasting plasma homocysteine concentrations ranged from 6 to 19 μmol/l, and postmethionine plasma homocysteine concentrations from 20 to 55 μmol/l. The 97.5 percentile levels of fasting and postmethionine plasma homocysteine in controls were calculated as 15 and 51 μmol/l, respectively. In the study group, fasting plasma homocysteine concentrations varied from 6 to 36 μmol/l, and postmethionine plasma homocysteine concentrations from 16 to 97 μmol/l. Hyperhomocysteinemia, i.e. fasting plasma homocysteine >15 μmol/l and/or postmethionine plasma homocysteine >51 μmol/l, was present in four of 46 (9%) controls and 26 of 84 (31%) women of the study group (Table 1; uncorrected chi-square, 8.3; $P < 0.05$).

The median concentrations of plasma homocysteine and blood vitamins are presented in Table 2. Median fasting and postmethionine plasma homocysteine were significantly higher in the study group as compared to the control group. The median concentrations of the vi-

Table 1
Prevalence of hyperhomocysteinemia in women of the control and study group as related to the level of fasting and postmethionine plasma homocysteine

Fasting plasma homocysteine	Postmethionine plasma homocysteine	Control group ($n = 46$)	Study group ($n = 84$)
Normal	Normal	42 (91%)	58 (69%)
High	Normal	3 (7%)	11 (13%)
Normal	High	1 (2%)	5 (6%)
High	High	0 (0%)	10 (12%)

Values represent numbers (proportions).

Table 2
Concentrations of plasma homocysteine and blood vitamins in women of the control and the study group

	Control group ($n = 46$)	Study group ($n = 84$)	P-value[a]
Fasting plasma homocysteine (μmol/l)	9 (6–19)	11 (6–36)	<0.05
Postmethionine plasma homocysteine (μmol/l)	29 (20–55)	37 (16–97)	<0.05
Serum folate (nmol/l)	14 (7–25)	12 (3–35)	<0.05
Red cell folate (nmol/l)	500 (310–1000)	510 (150–1300)	0.95
Serum vitamin B12 (pmol/l)	270 (100–580)	230 (60–620)	<0.05
Whole blood PLP (nmol/l)	53 (27–160)	42 (18–85)	<0.05

Values represent medians (minimum–maximum ranges). PLP, Pyridoxal-5′-phosphate.
[a]Wilcoxon rank sum test.

tamins studied were significantly lower in women of the study group as compared to the controls, except for red cell folate, where the median concentration was comparable in both groups.

In the control as well as study group, fasting and postmethionine plasma homocysteine were significantly and positively correlated ($r = +0.66$ and $r = +0.61$, respectively). Table 3 lists the associations between plasma homocysteine and blood vitamins in women of the control and study group. Fig. 4 depicts scatter diagrams of the concentrations of folate and vitamin B12 related to plasma homocysteine in women of the study group.

The median concentrations of fasting and postmethionine plasma homocysteine were not significantly different between the subgroups classified either by the type of the first event or the number of events (Table 4). The median concentration of fasting plasma homocysteine, unlike postmethionine plasma homocysteine, was significantly higher in women who experienced abruption or infarction in their first pregnancy than in women who had the same event after one or more uncomplicated pregnancies.

4. Discussion

The major finding of the present study is a high prevalence of hyperhomocysteinemia in women who experienced placental abruption or infarction. This result confirms an earlier preliminary report from our laboratory [12].

Placental abruption, a life-threatening event for the mother and her child, is thought to be the result of sudden rupture of the spiral artery. It often develops simultaneously with placental infarction which also markedly increases the risk of fetal or neonatal death [19]. Infarction of the placenta is predominantly the result of spiral artery occlusion in the myometrium or decidua. Histologic examination of the spiral arteries in placental infarction usually reveals one or more signs of vasculopathy, i.e. atherosis, narrowing, necrosis and thrombosis [19–23].

The hypothesis that elevated concentrations of plasma homocysteine affect the placenta is supported by the reported case of a woman with homocystinuria in whom four pregnancies resulted in intrauterine fetal death with multiple infarctions in the placenta [24]. As yet, the question how high levels of homocysteine may affect the spiral arteries is unanswered. Abnormalities of endothelial cells, platelets, clotting factors, serum lipids, or disorders in the complex interaction of these factors have been held responsible for the vascular damage and thrombogenesis in hyperhomocysteinemia [1,9,10]. In humans, the concentration of homocysteine in plasma is probably dependent on the extracellular homocysteine

Table 3
Associations between plasma homocysteine and blood vitamins in women of the control and study group

	Control group ($n = 46$)		Study group ($n = 84$)	
	Fasting plasma homocysteine	Postmethionine plasma homocysteine	Fasting plasma homocysteine	Postmethionine plasma homocysteine
Serum folate	−0.43†	−0.48†	−0.57†	−0.41†
Red cell folate	+0.07	+0.11	−0.47†	−0.24†
Serum vitamin B12	+0.08	+0.07	−0.35†	−0.18
Whole blood PLP	+0.03	+0.04	−0.11	−0.11

Values represent Spearman's rank correlation coefficients. PLP, Pyridoxal-5′-phosphate.
†Statistically significant ($P < 0.05$).

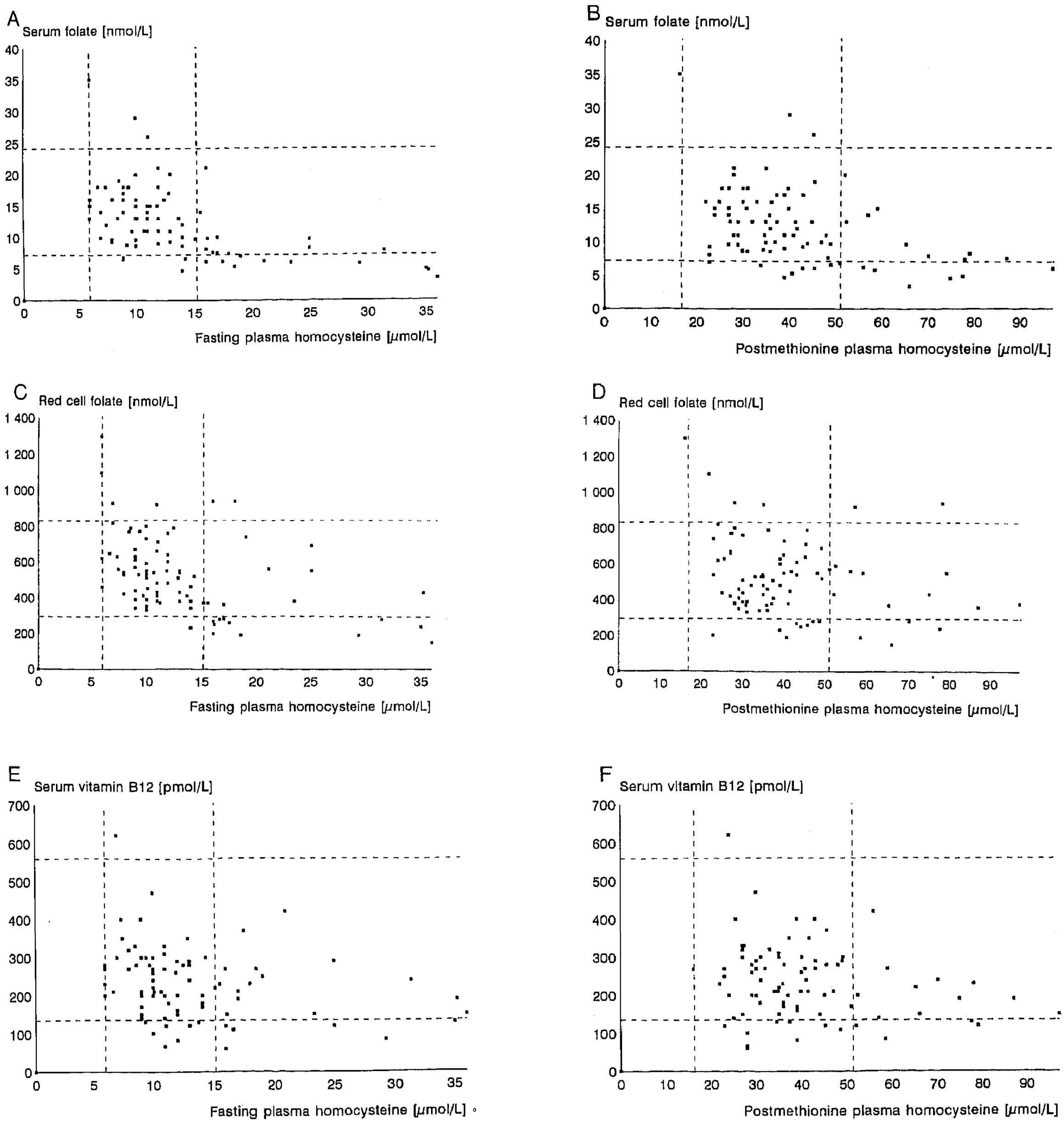

Fig. 4. Individual concentrations of blood vitamins (serum and red cell folate, and serum vitamin B12), and fasting and postmethionine plasma homocysteine in women of the study group. The dotted lines indicate the estimated 2.5 and 97.5 percentile levels of the control group.

export and on the capacity of homocysteine degradation in mainly the liver and kidney [25]. In endothelial cells in vitro, a delicate equilibrium exists between homocysteine export and degradation. Any disequilibrium resulting in hyperhomocysteinemia may contribute to the vulnerability of the endothelial cells [26].

Hyperhomocysteinemia may result from disorders in the transsulfuration or remethylation of homocysteine [1]. Vitamin deficiencies due to malabsorption or malnutrition, or enzymatic defects may interfere with both routes [2,3]. Hibbard (1964) has already suggested a higher prevalence of defective folate metabolism in women with placental abruption compared to controls, as indicated by their excessive formimino-glutamic acid

Table 4
Concentrations of fasting and postmethionine plasma homocysteine in women of the study group as classified by the type and pregnancy number of the first event, and the numbers of events

	Fasting plasma homocysteine (μmol/l)	Postmethionine plasma homocysteine (μmol/l)
Type of first event		
Abruption ($n = 44$)	12 (7–36)	38 (23–97)
Infarction ($n = 40$)	11 (6–35)	36 (16–87)
P-value[a]	0.28	0.60
Pregnancy number (G) of first event		
G = 1 ($n = 46$)	12 (6–36)	39 (23–87)
G > 1 ($n = 38$)	10 (6–29)	35 (16–97)
P-value[a]	0.04	0.13
Number of events		
One ($n = 66$)	11 (6–36)	38 (22–97)
Two or more ($n = 18$)	13 (6–35)	36 (16–78)
P-value[a]	0.21	0.46

Values represent medians (minimum–maximum ranges).
[a]Wilcoxon rank sum test.

excretion after histidine loading [27]. In the present study, the median levels of serum folate, serum vitamin B12 and whole blood PLP were significantly lower in women of the study group compared to those of the control group (Table 2). In addition, serum and red cell folate were observed to be significantly and negatively associated with plasma homocysteine (Table 3). We do not exclude the possibility that placental abruption or infarction, at least in some cases, result from a primary nutritional deficiency of vitamin B12 and/or folate, of which hyperhomocysteinemia is merely a concomitant finding.

Recently, a common mutation in the coding sequence of MTHFR was demonstrated to result in reduced MTHFR activities and increased plasma homocysteine concentrations in vascular patients [28]. Future studies should explore this mutation in women with placental abruption or infarction.

In the present study, the median concentration of fasting plasma homocysteine, unlike postmethionine plasma homocysteine, was significantly higher in women who experienced abruption or infarction in their first pregnancy than in women who had the same event after one or more uncomplicated pregnancies (Table 4). It is speculated that higher levels of plasma homocysteine result in placental malfunction earlier in maternal life. It can be argued, however, whether the difference in median plasma homocysteine concentrations of only 2 μmol/l will be of clinical significance.

Pyridoxine and/or folic acid administration have been reported to reduce plasma homocysteine concentrations in vascular patients with hyperhomocysteinemia [29–32]. It is not known whether biochemical normalization of hyperhomocysteinemia by periconceptional folate administration will favour pregnancy outcome in women with placental abruption or infarction. A randomized controlled prevention trial should provide the answer to this important question.

In conclusion, hyperhomocysteinemia is associated with placental abruption or infarction.

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